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Stratification and Quality Assessment of *Abies lasiocarpa* Seeds

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Stratification and Quality Assessment of *Abies lasiocarpa* Seeds

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ABSTRACT

Several seed sources of *Abies lasiocarpa* were subjected to various stratification treatments. Germination, moisture content, respiration, and protein content were monitored during stratification and incubation at three thermoperiods (15°C/10°C, 25°C/15°C, and 30°C/20°C). Germination was greatest when moisture content was reduced to 30-35% during the stratification period (stratification-redry treatment). Germination was not enhanced by the stratification-redry treatment in the nondormant and poor quality seedlots, but the seeds were less subject to mold during chilling.

Among the three thermoperiods, germination was lowest when seeds were incubated at 15°C/10°C. Under this cool regime, the best response was achieved when seeds received the stratification-redry treatment. At 30°C/20°C, seeds also germinated best when given the stratification-redry treatment. However, if seeds were incubated at 25°C/15°C, they germinated as well with 8-week stratification as with stratification-redry.

Seed performance was related to seed respiration and storage protein levels. Potentially, both measures could be developed as quantifiable indices of seed vigour. All seed sources exhibited significantly lower respiration rates during stratification and incubation when given the stratification-redry treatment. Total storage protein did not change appreciably during stratification, but tended to be significantly lower in poor quality seeds.

APPLICATION

The results of this study will be used in forest nurseries to:

1. improve techniques for releasing seeds from dormancy;
2. increase germination vigour;
3. optimize environmental conditions during germination; and
4. improve the ability of foresters to assess seed quality and vigour.

Key words: *Abies lasiocarpa*, seed dormancy, seed germination, seed protein, seed quality, seed respiration, subalpine fir, stratification, temperature

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INTRODUCTION

In the past several years there has been increased interest in subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) for reforesting northern, wet-belt, and high elevation sites in British Columbia. In north coastal locations such as the Bell-Irving River valley (latitude, 56°15'-56°30'; longitude, 129°30'-129°50'), where winter conditions are characterized by heavy snowfall, subalpine fir outperforms the white spruce (Picea glauca (Moench) Voss) usually grown in the area (Houseknecht *et al.* 1987).

About 5700 ha are harvested annually in the Bell-Irving area and, according to current silvicultural prescriptions for the area, more than 3 million *A. lasiocarpa* should be planted each year (G.F. Dodd, Kalum Forest District, pers. comm., 1987). However, Ministry and private nurseries have been unable to meet the desired seedling quotas for the species. Infrequent cone crops, poor seed quality, low germination, slow initial growth, and insufficient understanding of cultural requirements have impeded efforts to achieve desired seedling production at cost-effective levels.

The superior performance of *A. lasiocarpa* in cold, moist northern environments (Laing and McCulloch 1988) underlines the importance of improving our knowledge of the cultural requirements of this species. FRDA Project 2.41 dealt with the initial part of this cultural process, concentrating on the problems associated with seed dormancy and vigour.

The major portion of the study focused on seed treatments to break dormancy and to improve germination performance. Previous research indicated that stratification in which seed moisture content (mc) is controlled during the stratification period is very effective in breaking dormancy and increasing germination rates of *A. amabilis* (Dougl.) Forbes seeds (Leadem 1986). This modified type of stratification, referred to as the stratification-redry technique, has been found to be effective for a number of conifer seeds (Danielson and Tanaka 1978; Edwards 1980, 1981, 1986).

Experiments conducted under Project 2.41 during the fiscal year 1987/88 demonstrated that dormant seed sources of *A. lasiocarpa* also responded well to stratification-redry treatment (Leadem 1988). However, work previously reported on *A. amabilis* (Leadem 1986) showed that the effectiveness of stratification treatments can vary according to the temperature conditions at the time of germination.

In general, unstratified tree seeds germinate only within a relatively narrow range of temperatures. When they receive adequate stratification, however, they usually germinate over a much broader temperature range (Allen 1960; Adkins *et al.* 1984). Stratification also increases the rate of germination, so that seedling emergence is completed within a shorter period (Allen 1960, 1962; Edwards 1973). Stratification thus has two obvious benefits. It can be used to enhance seed germination, and to increase the number of temperature options available to nursery management.

Objectives

These experiments investigated the effects of stratification and temperature on germination of *Abies lasiocarpa* seeds, with the aim of demonstrating that seed germination is enhanced under low temperatures if seeds receive appropriate stratification treatment. Seed source was also considered as a factor in the experimental design because individual seedlots may respond differently, independent of the treatment.

The second objective of this study was to continue earlier work under Project 2.41 to develop some physiological indicators of seed vigour and seedling performance. Metabolic and biochemical indicators have been used with some success to evaluate vigour in agricultural seeds (Pollock and Roos 1972; Maguire 1977). In this study, vigour was defined as "those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (Bonner 1984).

Particular attention was given to seed respiration and protein content and how these parameters varied with different stratification and temperature treatments. Data obtained during the fiscal year 1987/88 showed that respiration was greater in *A. lasiocarpa* seeds when they were stratified at high mc (greater than 40%), rather than low mc (ca. 30%). As well, total protein content was lower in poor quality than high quality seeds. These results suggest that respiration and protein content could each have potential as indices of seed vigour and physiological status.

As part of the work in developing these methods, several questions were addressed in two supplementary experiments on seed respiration. These studies investigated the effects of temperature on the respiration rates of stratified and unstratified seeds, and the effects of seed coat removal on seed respiration. In addition, respiration of seeds with and without surface sterilization was compared to provide an indication of the degree of respiration attributable to microbes. All seeds sustain some level of fungal and bacterial contamination, especially in the outer seed coat. The contribution of microbial respiration to measured seed O_2 uptake must be determined if valid seed respiration rates are to be obtained.

MATERIALS AND METHODS

Seed Treatments

Experiment 1. *Stratification with moisture control (stratification-redry) and without.*

Three seed sources of *A. lasiocarpa* (Table 1: seedlots 3709, 3711, and 3714) received stratification treatments that varied from 0 to 24 weeks. The response of unstratified seeds (0 weeks) was used as a reference point to ascertain the degree of seed dormancy. Other stratification periods were chosen to compare the effects of length of stratification and of moisture level during the chilling period.

TABLE 1. *Abies lasiocarpa* seed sources

Lot #	Collection year	Seed zone	Latitude	Longitude	Elevation (m)
3709	1978	1070	50° 26'	122° 48'	1125
3711	1978	1150	55° 08'	127° 53'	495
3714	1978	5080	54° 56'	126° 42'	865
3717	1978	3080	52° 50'	119° 20'	838
28838	1986	1140	56° 10'	129° 20'	450

In the first six treatments, moisture was not reduced during stratification at 2°C (Table 2). Seeds were soaked for 48 hours, and seed moisture remained >45% mc during the entire chilling period (Fig. 1).

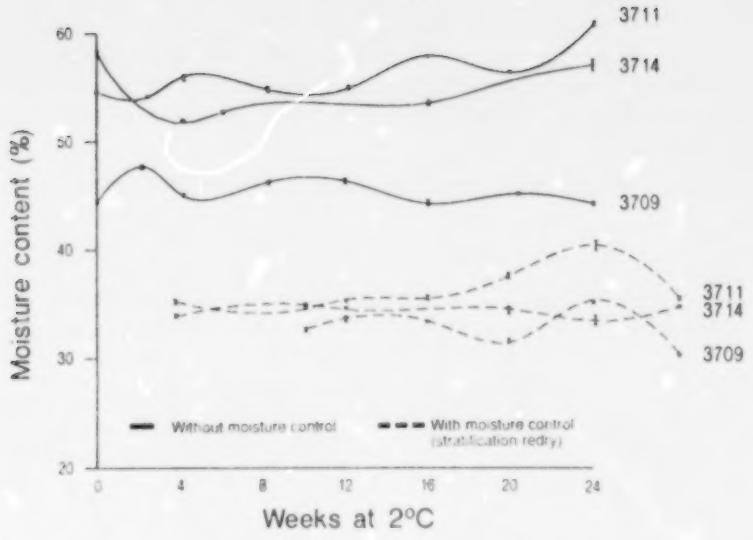

FIGURE 1. Moisture content of imbibed seeds during stratification with moisture control (stratification-redry) and without. Data are the means of two 0.5-g samples. Standard error bars have been given for each data point.

In the remaining four treatments, seed moisture was reduced to 35% mc after 4 weeks' stratification. For the stratification-redry procedure (treatment 9), seeds were soaked for 48 hours; then stratified for 4 weeks at 45% mc. After 4 weeks, seeds were removed from 2°C storage and air-dried on screens at 20°C for 4-8 hours until seed moisture reached 35% mc (fresh weight basis). Seeds were then returned to 2°C for an additional 12 weeks. In the remaining treatments (7, 8, and 10), the number of weeks at 35% was longer or shorter than in treatment 9. Plastic seed storage bags were loosely tied to permit air circulation during all stratification treatments.

TABLE 2. Stratification treatments of experiment 1

Treatment	Weeks	Stratification time (weeks) at 45% mc	Stratification time (weeks) at 35% mc
1	0	0	0
2	4	4	0
3	8	8	0
4	12	12	0
5	16	16	0
6	24	24	0
		Stratification-redry	
7	8	4	4
8	12	4	8
9	16	4	12
10	24	4	20

Experiment 2. *Stratification and incubation temperature*

Seeds from three seed sources (Table 1: seedlots 3714, 3717, and 28838) were soaked for 48 hours in de-ionized H_20. The water was changed after 24 hours to remove inhibitory substances which may have been leached from the seeds. After imbibition, seeds in each lot received three stratification treatments:

1. 0 stratification. Seeds were soaked, but received no chilling at 2°C.
2. 8-week stratification. Seeds were stratified at 2°C for 8 weeks at 40-50% mc (current Ministry of Forests Seed Centre practice).
3. Stratification-redry. Seeds were stratified at 2°C for 4 weeks (mc = 40-50%). After 4 weeks, seeds were removed from 2°C storage and air-dried on screens at 20°C for 4-8 hours until seed moisture reached 35% mc (fresh weight basis). Seeds were then returned to 2°C for an additional 12 weeks.

Treatments for each seedlot were started 1 week apart to distribute the workload. After stratification, seeds were incubated for 4 weeks in controlled environment chambers maintained at three different thermoperiods (30°C/20°C, 25°C/15°C, and 15°C/10°C). Light was given continuously during the 8-hour high temperature period.

Seed Assessment

Moisture content

Seed mc was determined on a fresh weight basis before and after the 48-hour soak, before and after the redry step, and at 4-week intervals during stratification. Measurements were made on two 0.5-g samples which were weighed before and after drying at 100°C for 24 hours.

Germination

Six replications of 50 seeds were used for each treatment. After treatment, seeds were incubated in controlled environment chambers at 30°C/20°C with the high temperature during the 8-hour light period. Seeds were placed in covered plastic boxes (12 X 12 X 3 cm) on a substrate of one layer of Kimpak and two layers of Whatman No. 1 filter paper, moistened with 50 mL de-ionized water. Light intensity was about 50 microeinsteins m^{-2} sec^{-1}. Germination counts were made on Monday, Wednesday, and Friday of each week and terminated after 4 weeks. Seeds were considered germinated once the radicle and hypocotyl had grown to 4 times the length of the seed coat.

Respiration

The respiration of seeds was monitored with a Clark oxygen electrode apparatus (Yellow Spring Instruments, Yellow Springs, Ohio). The monitoring system consists of a water bath assembly, four 20-mL cuvettes, an oxygen electrode, a constant temperature circulator, and a recorder. The oxygen electrode was equipped with a high sensitivity membrane to determine rates at temperatures below 20°C.

Seeds were placed in the cuvette with a phosphate buffer to retain peak physiological activity. Cuvette temperature was maintained with a constant temperature water bath assembly. The oxygen electrode is mounted in a tightly fitting plexiglas probe, which was milled to exclude all free oxygen when the probe was inserted down to the surface of the solution. Depletion of the oxygen within the cuvette was recorded with an analog monitor.

Respiration measurements were made throughout the stratification (Table 2) and germination periods (Table 3). Determinations were performed on samples of 10 seeds, and replicated 3 times. The integument (papery outer coat) was removed before respiration measurements were made, to reduce the amount of debris in the cuvettes. The seeds were covered with 3 mL of phosphate buffer (pH 7.5), and allowed to equilibrate for 3 minutes. Respiration was monitored for 5 minutes.

TABLE 3. Germination classes

Class	Description
1	No evidence of germination
2	Coat split ("chitted")
3	1 - 4 mm radicle
4	10 - 15 mm radicle
5	25 - 40 mm radicle (4X coat)

To determine the effects of temperature on respiration, the waterbath was maintained at 10°C, 20°C, and 30°C. Unstratified and stratified (8 weeks and stratification-redry) seeds from three seedlots were used to determine if differences in respiration levels were related to seed source.

To determine the effects of the seed coat on respiration of *A. lasiocarpa*, seeds from two seedlots were used. Three samples of 10 seeds (with and without seed coats) were removed from each stratification treatment. The seeds were surface-sterilized by immersion in full strength NaOCl (5.25% v/v) for 5 minutes, followed by three rinses with deionized water.

Seed storage protein

Seed samples were removed for protein determinations during stratification at 2-week intervals, or according to germination class (Table 3). Storage protein was determined on three samples of 10 seeds taken from each treatment. Seed coats were removed and samples were either ground in phosphate buffer (pH 7.5) before freezing, or frozen whole at -20°C. Extractions of total protein, soluble protein, and insoluble protein were performed as described in Appendix 1.

Experimental Design

Germination tests were performed in controlled environment chambers, with treatments arranged according to a completely randomized design. In experiment 1, three different seed sources of *A. lasiocarpa* were given 10 different treatments, and each seedlot/treatment combination was replicated 6 times. For the incubation temperature studies (experiment 2), six replications of each seedlot and stratification combination were randomized within three separate controlled environment chambers set at different thermoperiods. However, temperature treatments were confounded with chambers because only a single chamber was used per temperature regime. In all experiments, protein content and respiration measurements were replicated 3 times, and moisture contents were replicated twice.

RESULTS AND DISCUSSION

Moisture Content

After 48-hour imbibition, seeds attained 45 - 58% moisture, depending on the seedlot (Fig. 1). If seeds were not dried after 4 weeks' stratification, seed mc remained at initial levels throughout the 24-week stratification period. The mc of seeds dried after 4 weeks' stratification maintained about 30% throughout the treatment period.

Germination

The three *A. lasiocarpa* seed sources responded very differently to stratification-redry treatment (Fig. 2). Seedlot 3709 was considered a nondormant lot, since it germinated fairly well even without stratification and showed no marked response to redry treatment. It should be noted, however, that stratification-redry treatment was not detrimental to this nondormant seedlot. This may be an important consideration in operational situations where it is only feasible to apply a single seed treatment before sowing.

Seedlot 3714 showed the response of a dormant, high-quality lot. Eight weeks' stratification at high mc was not effective in breaking dormancy, and this lot had only 25% germination. Following stratification-redry treatment, this lot germinated 86%.

Seedlot 3711 did not germinate well, regardless of treatment. After 16 weeks' stratification at 2°C without moisture control, only 5% of the seeds had germinated, and all remaining seeds were infected with mold.

For many true firs, control of seed moisture during stratification enhances the effectiveness of stratification treatment (Edwards 1981; Leadem 1986). In all three *A. lasiocarpa* seedlots, higher germination was also achieved when seeds were stratified for 16 weeks at 35%, compared to seeds stratified for 16 weeks at 45%. This was generally true for all other stratification periods (8, 12, and 24 weeks) when comparisons are made between the germination of seeds stratified at 35% mc and those stratified at 45% mc.

Seed germination varied according to the temperature at which seeds were incubated, but the stratification treatment given before incubation altered the magnitude of the response (Fig. 3). Among the three thermoperiods, germination was lowest when seeds were incubated at 15°C/10°C. Under this cool regime, the best response for all three lots (54% average germination) was achieved when seeds received the stratification-redry treatment. By comparison, mean germination of all three lots after 8 weeks stratification was only 21%.

The stratification-redry treatment enhanced germination in the two warmer (25°C/15°C and 30°C/20°C) regimes as well. Mean germination of all three lots after stratification-redry was 82% and 78% at 25°C/15°C and 30°C/20°C, respectively. Seeds given 8 weeks' stratification only germinated 71% and 57% at 25°C/15°C and 30°C/20°C, respectively.

These results emphasize the importance of matching the dormancy-release treatment to the species. When seeds are appropriately stratified, germination is enhanced in several ways. Germination is not only greater, but seeds germinate more rapidly. Stratification has also been found to expand the range of temperatures under which germination occurs (Allen 1960; Adkins *et al.* 1984; Leadem 1986). Complete germination is usually expected at 30°C/20°C, but with the stratification-redry treatment, better than expected germination was shown at 15°C/10°C. This enhancement of germination has important ramifications for nurseries, which frequently experience low temperatures at the time of sowing.

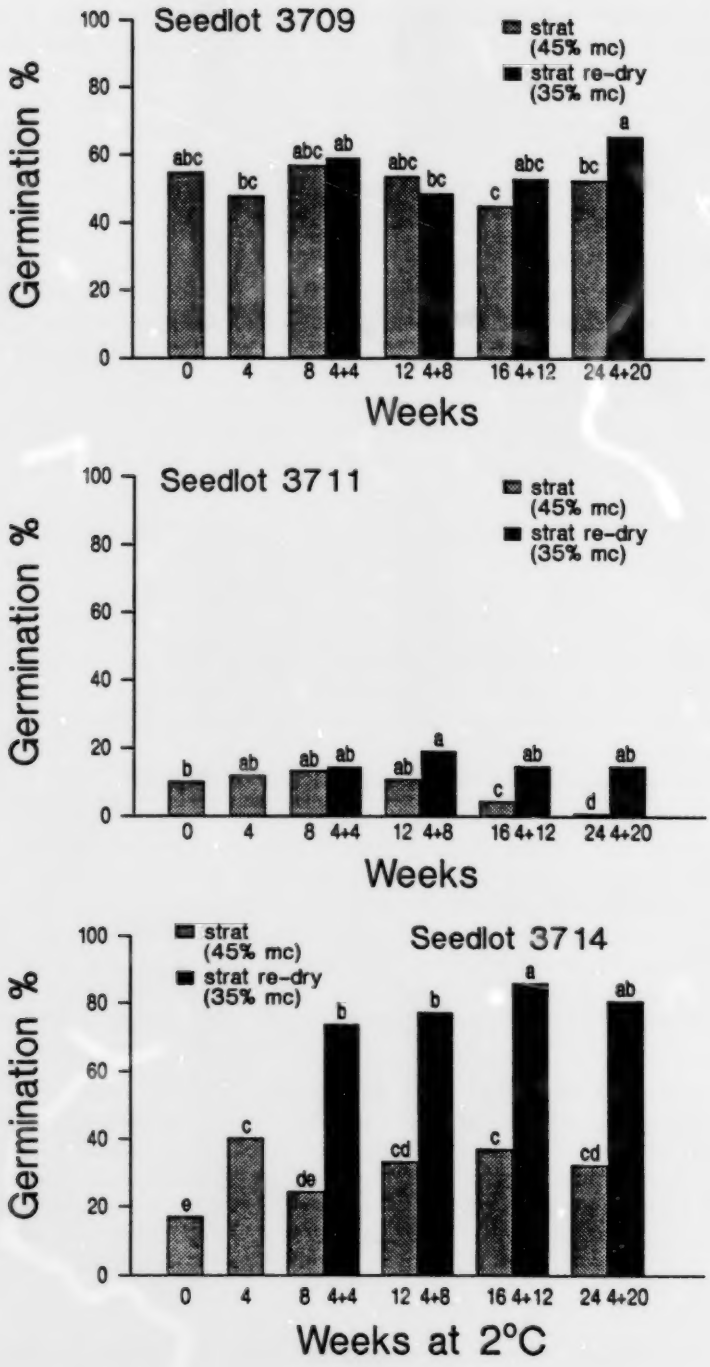

FIGURE 2. Effects of stratification treatment on the germination of three seed sources of *Abies lasiocarpa* after 28 days. Data are the means of six replicates of 50 filled seeds. For each seedlot, treatments with the same letter are not significantly different at p=0.05 as determined by Duncan's multiple range test (1965).

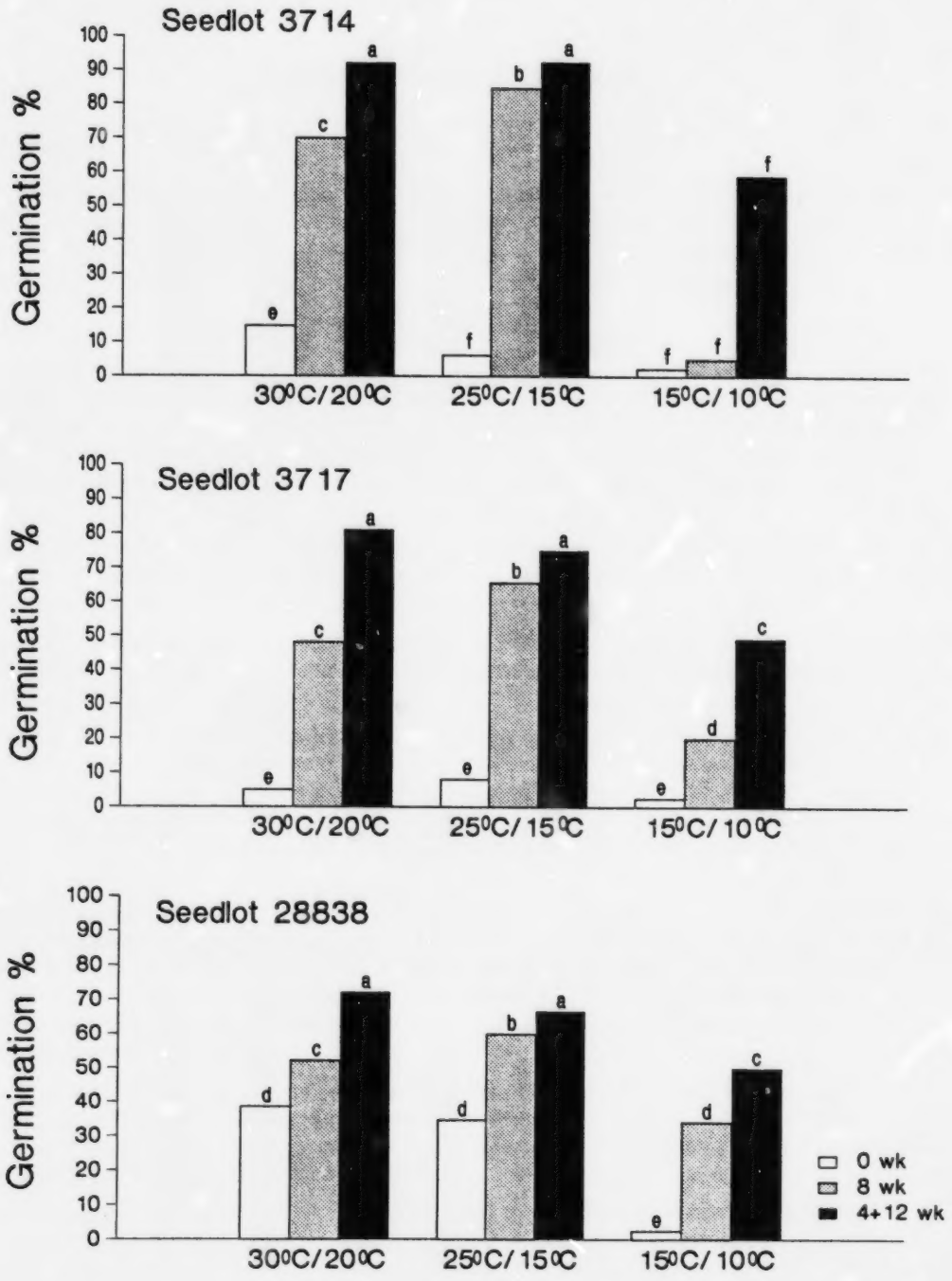

FIGURE 3. Effects of stratification treatment and incubation temperature on the germination of three seedlots of *Abies lasiocarpa* after 28 days. Germination tests were performed at the thermoperiods indicated on the figure, with 8 hours light given during the high temperature period. Data are the means of six replicates of 50 filled seeds. For each seedlot, treatments with the same letter are not significantly different at p=0.05 as determined by Duncan's multiple range test (1965).

The response of *A. lasiocarpa* to lower incubation temperatures has implications for seed testing protocols. International seed testing rules (ISTA 1985) prescribe thermoperiods of 30°C/20°C for testing most British Columbia conifer seeds, including *Abies*. Yet, the results presented in Figure 3 and elsewhere (Leadem 1986) indicate that *Abies* seeds given the usual stratification treatment (without moisture control) germinate better under slightly cooler temperatures (25°C/15°C). Since the Association of Official Analysts has revised its thermoperiod protocol for *A. amabilis* to 25°C/15°C (AOSA 1981), a review of incubation test conditions for all *Abies* species should also be considered in British Columbia.

Respiration

Seeds generate energy for growth and development by respiring stored reserves (Bewley and Black 1982; Ross 1984). The rate at which seeds use their reserves is an indication of their physiological efficiency and, potentially, also a measure of vigour. Data obtained for *A. lasiocarpa* during stratification indicate a possible relationship between physiological respiration and seed performance. In seedlot 3714, a good-quality but dormant lot, seed respiration remained relatively low throughout the chilling period when moisture was controlled (stratification-redry treatment) (Fig. 4). Respiration rates were also initially low in seeds stratified at high moisture levels (>45% mc), but they increased exponentially after 16 weeks.

A different pattern was observed in the low germinating lot 3711. Although respiration rates were relatively low for the first 4 weeks, seeds stratified at high mc exhibited respiration when the treatment continued past 4 weeks. By comparison, the respiration rates of seeds which had received stratification-redry treatment increased much more slowly during the chilling period.

The effects of stratification and temperature on seed respiration are shown in Table 4. Respiration is known to be strongly temperature-dependent (Salisbury and Ross 1969), and the effects of temperature are clearly seen in the response of *A. lasiocarpa* seeds. Respiration rates increased in all three lots as temperature was increased from 0 to 30°C. In two of the three lots, respiration was also higher at all temperatures in seeds that received 8 weeks stratification, compared to those that received stratification-redry treatment.

Microbial respiration is present in all natural systems, including seeds. The contribution of microbial respiration was addressed in an experiment that compared the effects of surface sterilization on total apparent respiration (Table 5). No significant differences were noted between seed treatments with or without surface sterilization. These data indicate that the measured apparent respiration is due primarily to the respiration of living seed tissues, and that respiration rates are not significantly affected (in healthy seeds) by microbes naturally present in the coats. However, in future studies, respiration rates of seeds known to be infected with pathogens should be compared with sterilized controls to determine the magnitude of microbial respiration.

Major differences were found between treatments in which seed coats were removed or left on the seeds (Table 5). Seed respiration was significantly higher in seeds with coats removed. Respiration usually increases when the radicle emerges through the coat, or seed coats are manually removed, because seed coats are relatively impermeable to oxygen (Come and Tissaoui 1973; Bewley and Black 1978). Removal or cracking of the coat increases the availability of oxygen, and results in increased respiration.

TABLE 4. Effects of stratification and temperature on respiration (μL min^{-1} g^{-1} dry weight) of *Abies lasiocarpa* seeds

Treatment	0°C	10°C	20°C	30°C
		Seedlot 3714		
Dry seeds	-	0.27c	0.62c	0.88b
48-h soak	0.59	1.04b	1.77a	3.07a
8-wk strat	0.30	1.45a	1.85a	2.85a
Strat-redry	-	0.47c	1.16b	2.49a
		Seedlot 3717		
Dry seeds	-	0.15b	0.32b	1.14b
48-h soak	-	0.68a	1.64a	2.50a
8-wk strat	0.79	0.80a	1.79a	2.48a
Strat-redry	-	0.77a	2.00a	2.80a
		Seedlot 28838		
Dry seeds	-	0.52c	1.08b	1.47b
48-h soak	-	1.40ab	1.93a	3.54a
8-wk strat	-	1.79a	1.95a	4.68a
Strat-redry	-	0.85bc	1.54ab	3.22a

Means with the same letter in the same seedlot and column are not statistically different at p = 0.05 when evaluated by Duncan's multiple range test (1965).

TABLE 5. Effects of sterilization and coat removal on respiration (μL min^{-1} g^{-1} dry weight) of *Abies lasiocarpa* seeds

Treatment	Sterilized		Not sterilized	
	+ Coat	– Coat	+ Coat	– Coat
		Seedlot 3714		
Dry seeds	0.86b	3.64b	0.88c	2.68c
48-h soak	2.74a	5.80a	3.40a	7.75a
8-wk strat	2.47a	7.46a	2.85ab	6.46b
Strat-redry	2.63a	7.20a	2.49b	5.88b
		Seedlot 3717		
Dry seeds	1.06c	4.75b	1.14c	1.72b
48-h soak	3.56a	7.60a	3.45a	8.10a
8-wk strat	2.23b	7.08a	2.48b	6.22a
Strat-redry	1.91bc	7.76a	2.80ab	6.84a

Means with the same letter in the same seedlot and column are not statistically different at p = 0.05 when evaluated by Duncan's multiple range test (1965). All measurements were made at 30°C.

Most of the respiration rates reported here were measured at 30°C, but responses at this temperature are not necessarily an indication of how seeds respond during stratification at 2°C. Although few comparisons have yet been made, the data suggest there is a relationship between respiration and seed performance which may partly explain how the stratification-redry treatment improves seed performance.

Dormant species such as the true firs require long stratification periods to achieve optimal performance (Leadem 1986). The data in this study (Fig. 4) indicate that if seed moisture is kept at high levels, respiration tends to rise with increasing time of chilling at 2°C. Increased respiration during stratification may significantly affect subsequent seed performance, as reserves respired during stratification will not be available for use during germination. If, however, stored products are slowly respired during stratification, more energy supplies will be available for the critical emergence and establishment period.

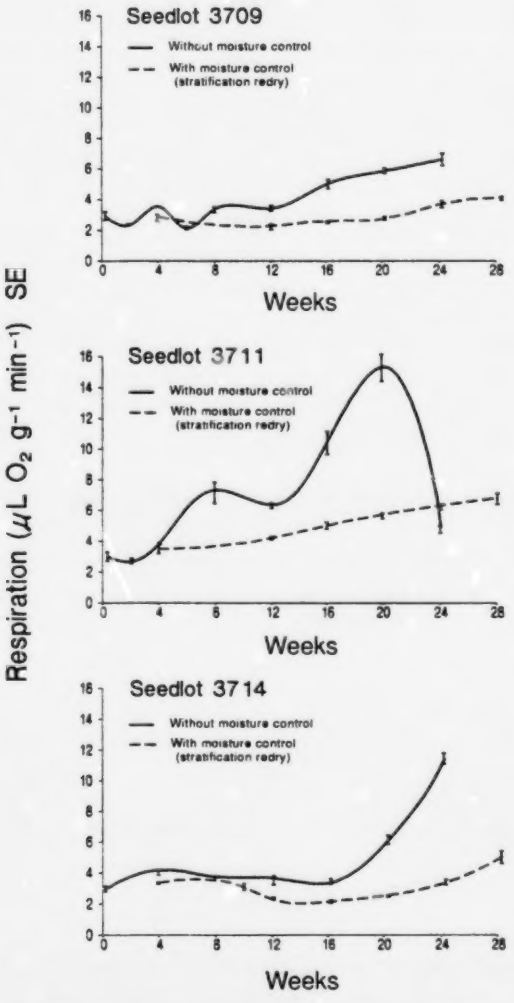

FIGURE 4. Seed respiration during stratification, with moisture control (stratification-redry) and without. Data are means of three replicates of 10 seeds, and standard error bars have been given for each data point. All measurements were made at 30°C.

Some support for this view was recently given in a study of sugar pine seeds in which a relationship was shown between high seed respiration rates and poor germination performance (Murphy and Hammer 1988). Changes in seed respiration may possibly also reflect changes in dormancy status. If respiration rates in Figure 4 are compared to germination data in Figure 2, it can be seen that the rise in respiration at approximately 16 weeks coincides with the length of stratification necessary to overcome the dormancy of the seedlot. This rise in respiration may be related to the breaking of seed dormancy.

The ability to identify when the breaking of dormancy occurs could prove to be valuable for assessing the physiological status of seeds. The usual method of determining stratification requirements is to subject seeds to a series of chilling times, and then germinate the seeds. The use of seed respiration as an alternative method of assessing performance would greatly simplify the search for optimal stratification treatments, since seed respiration can be measured with the Clark oxygen electrode in 5 minutes as opposed to the 3-4 weeks required for a germination test.

Seed Storage Protein

Germination depends solely on the levels of the reserves stored in the seeds (Bewley and Black 1978). Since conifer seeds store reserves mostly in the form of fats and protein, the amount of seed storage products would likely directly affect seed germination and initial seedling performance (Brooker *et al.* 1977; Tao and Khan 1977; Ross 1984).

Protein determinations were made on three seedlots at various times during stratification, but protein levels of individual seedlots did not vary significantly with time. Significant differences between seedlots did exist, however, in the mean total seed protein levels (Table 6, $F=23.39$, $P<0.001$). In addition, when germination and protein levels were compared, a relationship could also be seen between total protein and seedlot quality. Greater total protein values were associated with higher seed germination. These results are preliminary, but suggest a vigour index based on protein reserves might be developed. Ultimately, specific proteins might be found, whose presence is highly correlated with seed germination and lot quality, but further studies are necessary to determine the identity of the candidate proteins.

TABLE 6. Total protein and germination of *Abies lasiocarpa* seeds

Seedlot	Total protein conc. (mg/10 seeds)	Germination (%) (4 wk at 45% + 12 wk at 35%)
3714	26.16 a	86.0 a
3709	23.98 b	53.3 b
3711	18.56 c	14.7 c

Means with the same letter in the same column are not significantly different at p = 0.05 by Duncan's multiple range test (1965).

Seed Source

It is known that germination and other characteristics of individual seedlots of most conifers will vary, independent of the treatment being given. Thus, in most seed research, results are not extrapolated to the species level until a number of seed sources have been tested. Under Project 2.41, four of the five *A. lasiocarpa* seedlots tested responded positively to stratification-redry treatment, and the fifth lot exhibited such low germination that none of the treatments was effective. Although relatively few seedlots have been tested, statistical analysis has shown that stratification treatment had major effects on germination, and that variation due to seedlot was relatively minor.

Also, from extensive previous experience with *A. amabilis* (Leadem 1986; Leadem and Edwards[1]) and other *Abies* species (Edwards 1980, 1981, 1986; Tanaka and Edwards 1986; Y. Tanaka, pers. comm. 1982) it appears that *A. lasiocarpa* exhibits the generalized response of *Abies* species to stratification-redry treatment. The effectiveness of the treatment in enhancing germination speed and performance of *Abies* seeds under a range of temperatures in the laboratory and the field has been repeatedly shown in this and the several other studies cited above. It is therefore recommended that stratification-redry treatment be adopted as the standard procedure for operational stratification of all *Abies* species, preferably starting with the 1990 sowing season.

EXTENSION AND DEMONSTRATION

To realize the benefits of the improved stratification techniques described in this report, the Ministry of Forests Seed Centre must upgrade its systems for internal information management and process control. Implementing the stratification-redry technique means that the Centre must be able to capture and transfer raw data efficiently, because moisture content must be monitored often while seed moisture is being reduced during the drying step.

Several meetings have been held with Silviculture Branch staff to determine overall information system specifications for the Seed Centre. A schematic was prepared by Tree Seed Physiology staff, outlining all major operations of the Seed Centre and providing an overview of the relationships between various Centre operations. This preliminary plan identifies the points at which provisions should be made to interface the inputs and outputs of different operations with existing data management systems.

An automatic data capture system used by the Tree Seed Physiology laboratory was also recently demonstrated to Seed Centre personnel. The system uses an electronic balance with RS-232 output, a portable data logger, and a software program to transfer data to a personal computer. A comprehensive data handling system for entry and analysis of germination data was explained in detail. Full documentation of both systems and a copy of all relevant software have been given to Seed Centre staff.

Tree Seed Physiology staff are continuing to offer consulting services to the Seed Centre personnel to overcome any data handling and technical problems associated with the implementation of this stratification technique.

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1 Leadem, C.L. and D.G.W. Edwards. 1980. Unpublished data.

RECOMMENDATIONS

It is recommended that:

- the B.C. Ministry of Forests Seed Centre upgrade its internal information management and process control systems to realize the benefits of improved stratification techniques.

  Because seed moisture must be monitored frequently during the drying step, implementing the stratification-redry technique means that the Centre must be able to capture and transfer raw data efficiently.

- the B.C. Ministry of Forests Seed Centre adopt a protocol in which all *Abies* seedlots (*A. amabilis*, *A. lasiocarpa*, *A. grandis* (Dougl.) Lindl., and *A. procera* (Rehd.)) are tested both with and without stratification-redry treatment.

  Dormant seedlots benefit the most from stratification-redry treatment. The degree of dormancy of a seedlot is best derived from an examination of the results of paired germination tests, in which seeds receive or do not receive stratification. These data will indicate to Seed Centre staff which treatments are the most appropriate for preparing seeds for sowing when they are withdrawn from storage.

- seed germination testing thermoperiod protocols at the B.C. Ministry of Forests Seed Centre be changed from 30°C/20°C to 25°C/15°C for all *Abies* species.

  Maximum germination is obtained under slightly cooler thermoperiods. Testing at these temperatures will be a better indication of the potential of the seedlot.

- the stratification-redry treatment be applied, on an operational basis, to all seed sources of *Abies lasiocarpa*.

  Data obtained over the 2 years of this FRDA project complement the information obtained for other *Abies* species. Stratification-redry treatment enhances total germination, germination rate, and the ability of seeds to germinate at low temperatures.

- studies of the effects of temperature on germination be extended to include additional seed sources of *A. lasiocarpa* and other *Abies* species.

  A systematic survey of all *Abies* seedlots in storage, to evaluate their response to a range of germination temperatures, can be conducted by the Seed Centre or through contract research.

- research be continued on the relationship between seed respiration, storage protein, and seed performance with a view to developing alternative methods of assessing seed quality.

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APPENDIX 1. Experimental procedures: biochemical determinations protein extraction procedure (Gifford *et al.* 1982)

A. Total Protein

i) To 1.0 mL sample, add 1.5 mL 0.05 M potassium phosphate buffer (pH 7.5).

ii) Add 2.5 mL 0.065 TRIS/10% glycerol/2% SDS (pH 6.8).

iii) Heat in a boiling water bath for 15 minutes.

iv) Centrifuge at 40 000 X g for 30 minutes at 16°C (25 000 RPM with Type 40 rotor).

v) Remove supernatant and label tubes to be assayed for protein (A or B, 1-6, TP, date).

B. Soluble Protein

i) To 1.0 mL sample, add 4.0 mL 0.05 M potassium phosphate buffer (pH 7.5).

ii) Centrifuge at 40 000 X g for 30 minutes at 16°C.

iii) Remove supernatant and label tubes (A or B, 1-6, SP, date). Save pellets for insoluble protein.

C. Insoluble Protein

i) Re-suspend pellet from B in 2.5 mL potassium phosphate buffer (pH 7.5).

ii) Add 2.5 mL 0.065 TRIS/10% glycerol/2% SDS (pH 6.8).

iii) Heat in a boiling water bath for 15 minutes.

iv) Centrifuge at 40 000 X g for 30 minutes at 16°C.

v) Remove supernatant and label tubes (A or B, 1-6, IP, date).

Protein Assay (Lowry *et al.* 1951)

Standard Solution: 0.25 mg/mL bovine serum albumin

Reagents: (for 80 samples)

A 2% Na_2CO_3 in 0.1 N NaOH

B 2% NaK tartrate

C 1% $CuSO_4.5H_2O$

D Mix 2.5 mL (B) with 250 mL (A), add 2.5 mL (C), and mix well. Use within 2 hours.

E Folin-ciocalteau phenol reagent (9 mL). Dilute with an equal volume distilled H_2O. Use within 2 hours.

F (T + K solution). Mix 1 mL 0.05 M potassium phosphate buffer (pH 7.5).

Protocol for standards

Solution	Blank	1	2	3	4	5	T + K	
							Blank1	Blank2
Standard	0.0 mL	0.1	0.2	0.3	0.4	0.5	0.0	0.0
0.2N NaOH	0.5 mL	0.4	0.3	0.2	0.1	0.0	0.4	0.3
Solution D	2.5 mL	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Solution F	0.0 mL	0.0	0.0	0.0	0.0	0.0	0.1	0.2

Assay 0.1 mL and 0.2 mL aliquots of sample similarly.

After addition of (D) to each tube, vortex well and let mixture stand at room temperature for 10 minutes. Add 0.2 mL of (E) to each tube and mix immediately after each addition. Let tubes stand at room temperature for 30 minutes. Measure absorbance at 600 nm.

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